Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

Open a New Window on the World of Circulating microRNAs by Merging ChemiRNA Tech with Luminex Platform

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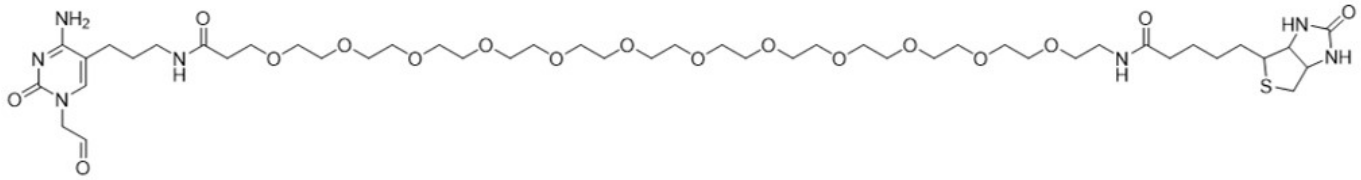
Electronic Supplementary Information (ESI)

Table S1 – Sequences

Sequence ID	Name	Peptide with abasic position (N'-C')
1	DGL 122	xx-CACCATT*GT*_AC*ACT*CCA
2	DGL 451	xx-AGT*AATGGT*AA_GGT*TT
3	DGL 193	xx-CTCGCCC*G_AA*AGACCCA
		miRNA sequence (5'-3')
4	Target miR-122-5p	UGGAGUGUGACAAUGGUGUUUG
5	Target miR-451a-5p	AAACCGUUACCAUUACUGAGUU
6	Target miR-193a-5p	UGGGUCUUUGCGGGCGAGAUGA

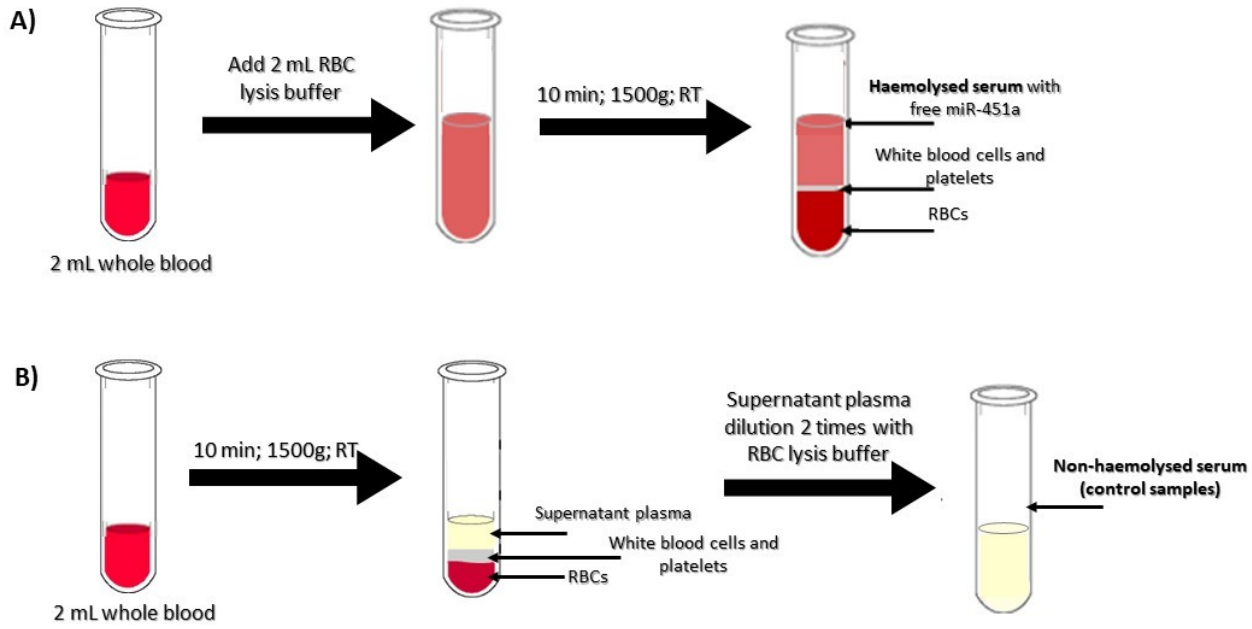
xx = amino-PEG-linker; "*" = propanoic acid side chain at the gamma position; "_" = abasic unit containing a propanoic acid side chain at the gamma position. The underlined sequence miR-122-5p, miR-451a-5p and miR-193a-5p are the regions that hybridise with the DGL 122, DGL 451 and DGL 193, respectively. The "G" in positions in bold are opposite to the abasic unit monomers and allows the specific incorporation of the aldehyde-modified biotinylated cytosine (Figure S1).

Figure S1 – Aldehyde-modified biotinylated cytosine



Chemical structure of aldehyde-modified biotinylated cytosine

Figure S2 – Preparation of serums with and without haemolyse



To obtain haemolysed serum, 2 mL of whole blood was incubated 10 min with 2 mL of RBC lysis buffer with a proportion of 1:2. The 4 mL solution was centrifuged at 1,500g for 10 min at RT to separate blood's figurative elements (pellet) from the supernatant serum which contains free miR-451a-5p. The supernatant was aliquoted into 0.2 mL Eppendorf and stored at -80 ºC; B) To obtain non-haemolysed serum, 2 mL of whole blood was centrifuged at 1,500g for 10 min at RT to separate the supernatant serum from the figurative elements of blood (pellet). The supernatant was collected and diluted 2 times with RBC lysis buffer. The diluted supernatant (non-haemolysed plasma) was aliquoted into 0.2 mL Eppendorf and stored at -80 C.

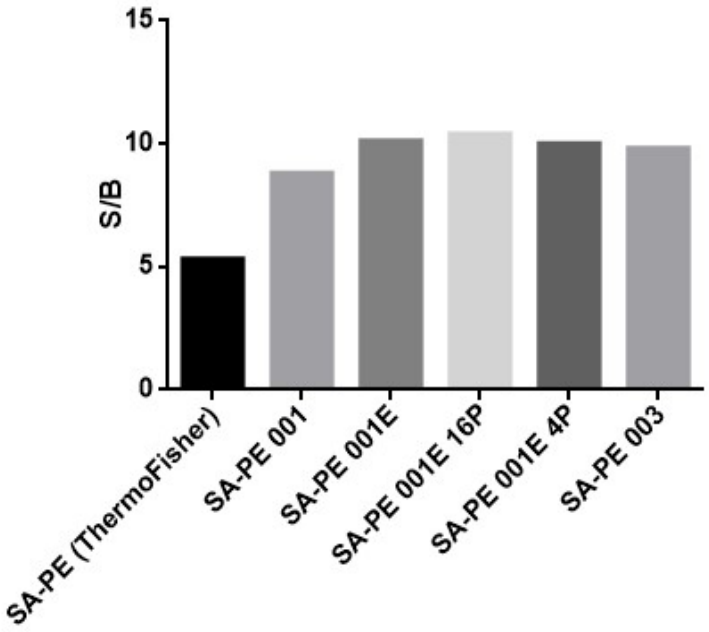
Table S2 -Serum pool samples.

ID sample	DILI (% volume)	HS (% volume)
1	75	25
2	50	50
3	25	75

Section S2 – Luminex MagPlex beads coupling with DGL-Probes

3 x 10^5 beads were resuspended in MES Buffer (50 µL, 0.1 M, pH 4.5) in an (size) eppendorf tube and combined with (i) a solution with 200 pmoles of DGL-probe (4 µL, 50 µM), (ii) EDC (2.5 µL, 10 mg/mL in deionised water). The solution was vortexed and incubated for 30 min at RT with shaking in darkness. 2.5 µL of a second EDC solution (10 mg/mL freshly prepared) was added, and the solution incubated for further 30 min at RT in darkness. Beads were washed first with 0.5 mL of 0.02% Tween 20 and second with 0.5 mL of 0.1% SDS. After washing, beads were resuspended and incubated with 200 µL of Ethanolamine (50 mM) in 0.1% Tween 20 (pH 8) for 1h, under vortexing at RT in darkness. The beads were washed one more time in 0.5 mL of 0.02% Tween 20 solution, and 0.5 mL of 0.1% SDS solution. Finally, the beads were resuspended in 240 µL of bead diluent.

Figure S3 – Assay performance using a selection of SA-PE(s)



The S/B ratio is the MFI signal obtained from "positive" wells containing 0.3 fmoles of complementary synthetic miR mimic miR-122-5p divided by the MFI signal obtained from the blank wells (negative). Positives and negatives were run in duplicates (n=2). SA-PE concentration was 2 µg/mL.

Table S3 – miR-122-5p MFI values measured in the singleplex sensitivity study

fmols	MFI average	CV	S/B
200.00	14801.50	2.69%	356.66
50.00	10573.50	1.61%	254.78
12.50	5430.50	2.64%	130.86
3.13	2138.50	0.76%	51.53
0.78	555.50	14.89%	13.39
0.20	161.50	2.19%	3.89
0.05	67.00	0.00%	1.61
Blank	41.50	1.70%	---
	Intra CV average	3.31%	

Experiments were performed in duplicate for each calibration point.

Table S4 – miR-451a-5p MFI values measured in the singleplex sensitivity study

fmols	MFI average	CV	S/B
200.00	11504.50	3.92%	92.04
50.00	7399.00	2.26%	59.19
12.50	2903.50	6.50%	23.23
3.13	1113.75	2.95%	8.91
0.78	465.00	3.65%	3.72
0.20	285.00	1.98%	2.28
0.05	181.50	4.29%	1.45
Blank	125.00	1.13%	---
	Intra CV average	3.34%	

Experiments were performed in duplicate for each calibration point.

Table S5 – miR-193a-5p MFI values measured in the singleplex sensitivity study

fmols	MFI average	CV	S/B
200.00	20360.50	1.48%	768.32
50.00	11306.50	6.09%	426.66
12.50	5413.00	2.27%	204.26
3.13	1595.75	3.66%	60.22
0.78	363.00	9.35%	13.70
0.20	160.25	0.22%	6.05
0.05	60.75	11.06%	2.29
Blank	26.50	2.67%	---
	Intra CV average	4.60%	

Experiments were performed in duplicate for each calibration point.

Section S3 – Curve equations

Standard curves were generated applying the Five Parameter Logistic (5PL) curve fit as shown below in 'a'. Data obtained for each standard curve is reported in the table 'b' below.

a. $Log_{10} X = Log\ EC50 + (1/HillSlope) * Log_{10} (2^{1/S}-1)$

b. Table:

	Log EC50	**Hill Slope**	**S**
miR-122-5p singleplex	1.540	0.786	1.190
miR-451a-5p singleplex	1.564	0.356	2.152
miR-193a-5p singleplex	3.655	0.239	3.264
miR-122-5p multiplex	1.790	0.480	4.844
miR-451a-5p multiplex	3.180	0.266	5.282
miR-193a-5p multiplex	1.718	0.567	3.830

Data were processed by GraphPad Prism 6.01 (GraphPad Software Inc., SanDiego, CA, USA).

Table S6 – miR-122-5p, miR-451a-5p and miR-193a-5p MFI values measured in the multiplex sensitivity study

fmols	MFI average			CV			S/B		
	miR-122-5p	miR-451a-5p	miR-193a-5p	miR-122-5p	miR-451a-5p	miR-193a-5p	miR-122-5p	miR-451a-5p	miR-193a-5p
200.00	15183.00	12123.25	19325.00	4.60%	0.39%	1.60%	326.52	102.31	743.27
50.00	10666.25	7178.50	13362.75	0.94%	0.05%	0.12%	229.38	60.58	513.95
12.50	5777.75	3554.50	6708.25	3.64%	12.99%	0.31%	124.25	30.00	258.01
3.13	2125.00	1172.75	2023.75	6.02%	1.66%	3.06%	45.70	9.90	77.84
0.78	532.00	520.75	506.25	5.75%	8.62%	3.14%	11.44	4.39	19.47
0.20	195.00	255.25	153.25	7.25%	4.02%	8.07%	4.19	2.15	5.89
0.05	64.50	168.50	70.50	3.29%	2.10%	2.01%	1.39	1.42	2.71
Blank	46.50	118.50	26.00	4.56%	2.98%	5.44%	---	---	---
		Intra CV average		4.60%	4.10%	2.97%			

Experiments were performed in duplicate for each calibration point.

Table S7 – Inter-assay variability

fmols	MFI average singleplex			MFI average multiplex			Inter-CV		
	miR-122-5p	miR-451a-5p	miR-193a-5p	miR-122-5p	miR-451a-5p	miR-193a-5p	miR-122-5p	miR-451a-5p	miR-193a-5p
200.00	14801.50	11504.50	20360.50	15183.00	12123.25	19325.00	1.80%	3.70%	3.69%
50.00	10573.50	7399.00	11306.50	10666.25	7178.50	13362.75	0.62%	2.14%	11.79%
12.50	5430.50	2903.50	5413.00	5777.75	3554.50	6708.25	4.38%	14.26%	15.11%
3.13	2138.50	1113.75	1595.75	2125.00	1172.75	2023.75	0.45%	3.65%	16.72%
0.78	555.50	465.00	363.00	532.00	520.75	506.25	3.06%	8.00%	23.31%
0.20	161.50	285.00	160.25	195.00	255.25	153.25	13.29%	7.79%	3.16%
0.05	67.00	181.50	60.75	64.50	168.50	70.50	2.69%	5.25%	10.51%
Blank	41.50	125.00	26.50	46.50	118.50	26.00	8.04%	3.78%	1.35%
					Inter CV average		4.29%	6.07%	10.70%

Experiments were performed in duplicate for each calibration point.

Table S8 – miR-122-5p, miR-451a-5p and miR-193a-5p MFI values measured in the multiplex study of clinical samples

Sample ID	MFI average			CV		
	miR-122-5p	miR-451a-5p	miR-193a-5p	miR-122-5p	miR-451a-5p	miR-193a-5p
DILI	3706.50	108.00	25.00	2.80%	10.48%	0.00%
PS 1	2928.14	4184.04	25.00	1.88%	0.09%	5.66%
PS 2	2112.71	5953.50	35.50	5.94%	12.10%	5.98%
PS 3	1221.00	7350.30	26.50	8.22%	6.04%	2.67%
HS	54.00	8367.75	29.00	1.90%	4.37%	3.98%

Experiments were performed in duplicate for each sample.

Table S9 – miR-122-5p, miR-451a-5p and miR-193a-5p fmols calculated in clinical samples

Sample ID	miR average quantity (fmols)			% of miR quantities as reported in Figure 3		
	miR-122-5p	miR-451a-5p	miR-193a-5p	miR-122-5p	miR-451a-5p	miR-193a-5p
DILI	6.27	n.d.	n.d.	100.00%	n.d.	n.d.
PS 1	4.58	17.12	n.d.	73.01%	21.84%	n.d.
PS 2	3.09	33.50	n.d.	49.24%	42.73%	n.d.
PS 3	1.71	52.31	n.d.	27.36%	66.72%	n.d.
Haemolysed serum	n.d.	78.40	n.d.	n.d.	100.00%	n.d.

Values of columns 2, 3 and 4 were calculated by extrapolating the fmols from the calibration curves reported in Figure 2B. Values of columns 5, 6 and 7 refer to the % of miRs. % are calculated as described in the main text.